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Date:

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of JC Xu et al.

Group Art Unit: 1642

Application No.: 09/030,606

Filed: February 25, 1998

For: COMPOSITIONS FOR IMMUNODIAGNOSIS OF PROSTATE CANCER AND METHODS FOR THEIR USE

Examiner: M. Davis

Docket No.: 210121.428c3

DECLARATION OF RAYMOND L. HOUGHTON, PH.D.

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D. C. 20231

The undersigned, Dr. Raymond Houghton, hereby declares:

1. I am a Senior Scientist at Corixa Corporation, the assignee of the subject application.

2. The following studies were carried out under my supervision.

To evaluate the presence of P501S in blood from prostate cancer patients, an immunocapture method was employed to first enrich for epithelial cells prior to quantitative real-time PCR analysis. Epithelial cells were enriched from blood samples taken from prostate cancer patients and from normal donors using the immunomagnetic bead separation method (Dynal A.S, Oslo, Norway). This technique utilizes a magnetic bead coated with monoclonal antibodies specific for glycopolypeptide antigens on the surface of human epithelial cells. Cells isolated this way were lysed and the magnetic beads removed. The lysate was then processed for poly A^+ mRNA isolation using magnetic beads (Dynabeads) coated with Oligo $(dT)_{25}$. After washing the beads in the

kit buffer, bead/polyA$^+$RNA samples were suspended in 10mM Tris HCl pH 8 and subjected to reverse transcription. The resulting cDNA was subjected to real-time PCR using P501S-specific primers and the Taqman™ procedure. β-Actin content was also determined and used for normalization. As shown in Figure 1 (attached), significantly higher quantities of P501S were found in blood samples from prostate cancer patients than in blood samples from normal donors.

The presence of P703P in blood obtained from two SCID mice implanted with prostate tumors (referred to as P93 and P63) and in blood obtained from one normal, control, mouse was evaluated as follows. Clotted mice blood was spun down and serum was harvested. RNA was isolated from the serum and subjected to reverse transcription using standard procedures. The resulting cDNA was subjected using real-time PCR using primers specific for P703P and standard protocols. As shown in the attached Figure 2, while the presence of P703P was detected in both the SCID mice blood samples, no P703P was detected in the blood sample from the normal mouse.

These results indicate that P501S and P703P may be effectively employed in diagnostic tests to detect the presence of prostate tumors.

3. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Raymond L Houghton

Raymond L. Houghton, Ph.D.

4/16/01

Date

Figure 1

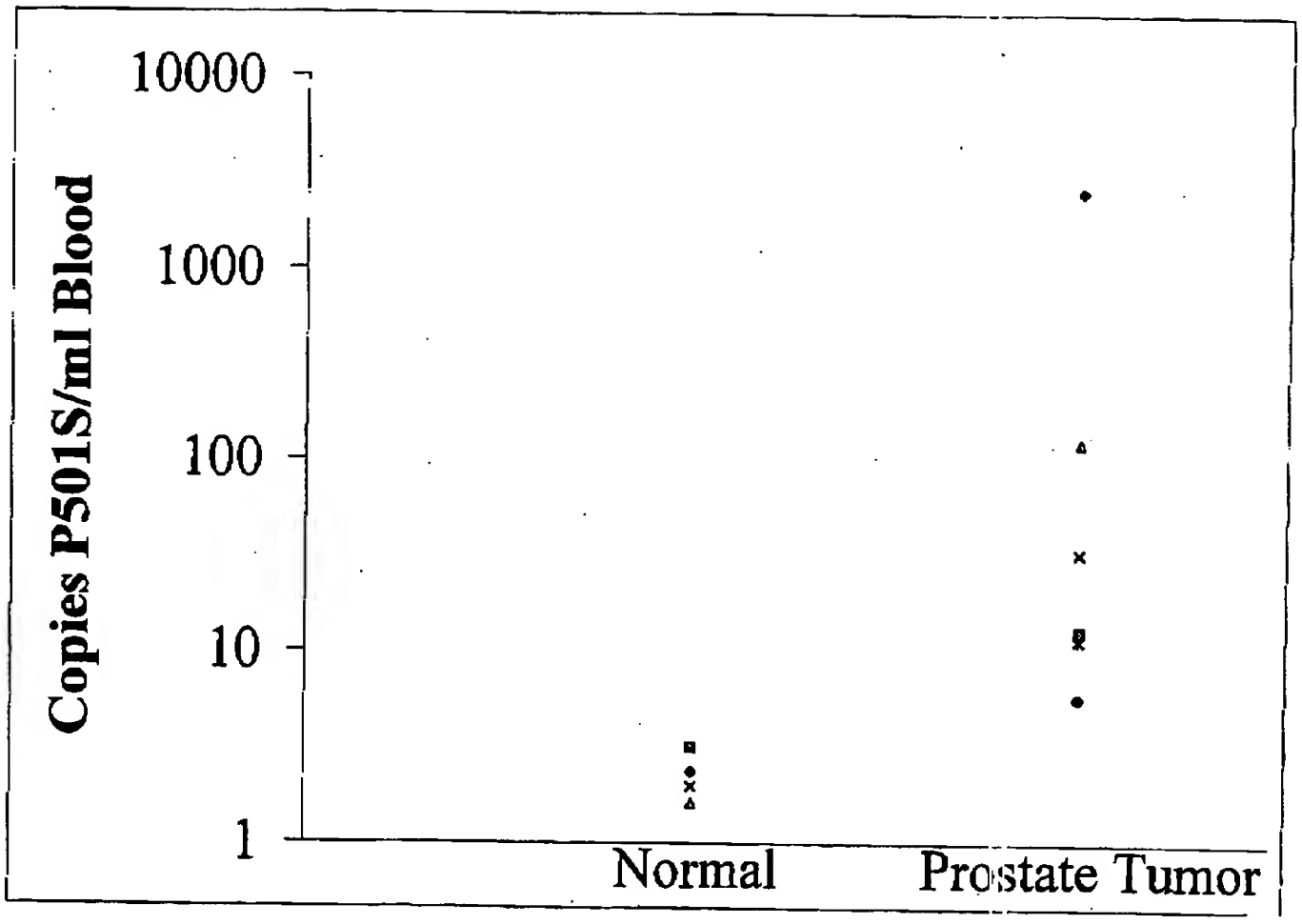
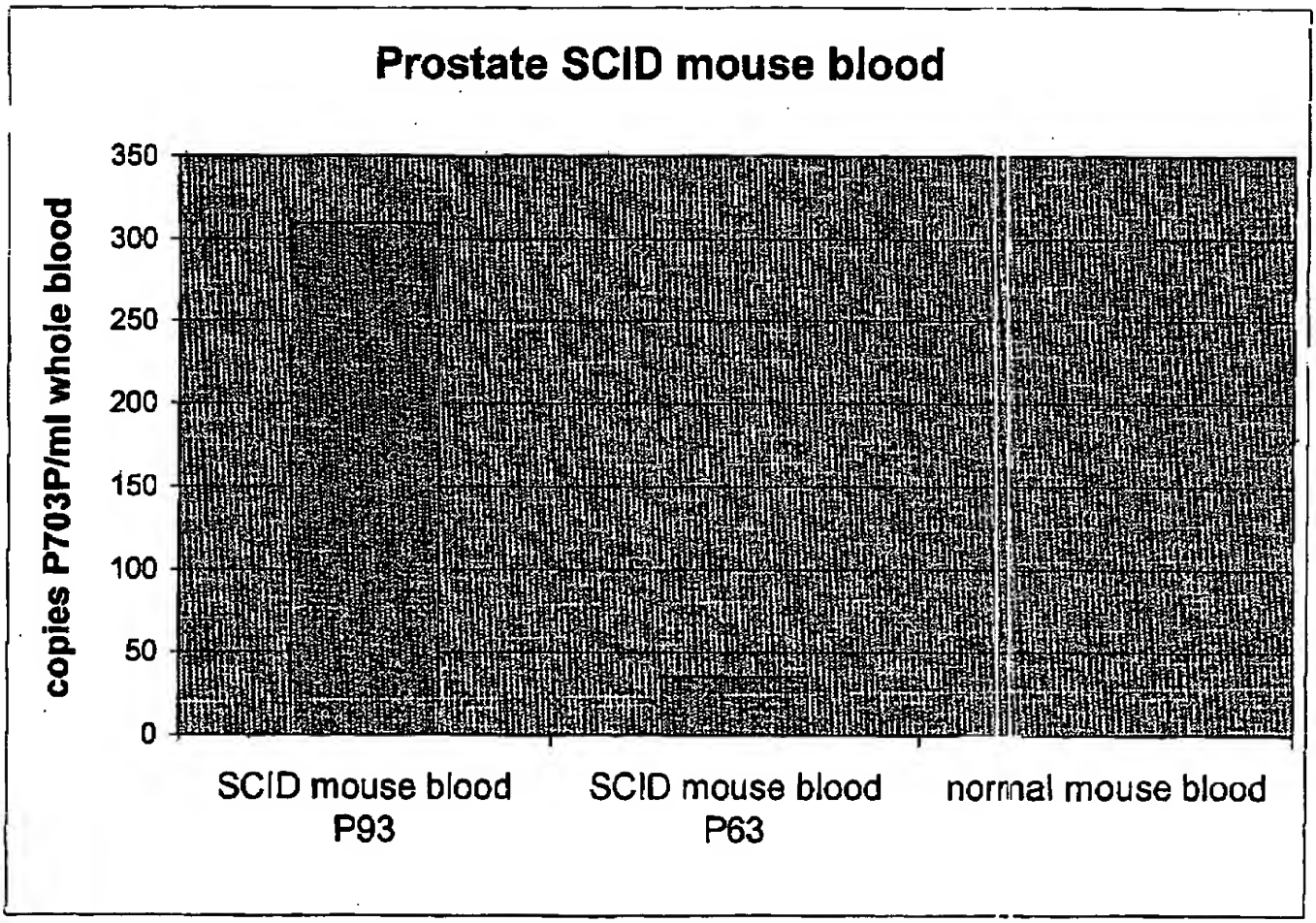

10000
1000
100
10
1
Copies P501S/ml Blood
Normal
Prostate Tumor

Figure 2


Prostate SCID mouse blood
copies P703P/ml whole blood
350
300
250
200
150
100
50
0
SCID mouse blood P93
SCID mouse blood P63
normal mouse blood